



# The New Biology of Gastrointestinal Hormones

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**Rehfeld, Jens F.** The New Biology of Gastrointestinal Hormones. *Physiol. Rev.* 78: 1087–1108, 1998.—The classic concept of gastrointestinal endocrinology is that of a few peptides released to the circulation from endocrine cells, which are interspersed among other mucosal cells in the upper gastrointestinal tract. Today more than 30 peptide hormone genes are known to be expressed throughout the digestive tract, which makes the gut the largest endocrine organ in the body. Moreover, development in cell and molecular biology now makes it feasible to describe a new biology for gastrointestinal hormones based on five characteristics. 1) The structural homology groups the hormones into families, each of which is assumed to originate from a common ancestral gene. 2) The individual hormone gene is often expressed in multiple bioactive peptides due to tandem genes encoding different hormonal peptides, alternative splicing of the primary transcript, or differentiated processing of the primary translation product. By these mechanisms, more than 100 different hormonally active peptides are produced in the gastrointestinal tract. 3) In addition, gut hormone genes are widely expressed, also outside the gut. Some are expressed only in neuroendocrine cells, whereas others are expressed in a multitude of different cells, including cancer cells. 4) The different cell types often express different products of the same gene, "cell-specific expression." 5) Finally, gastrointestinal hormone-producing cells release the peptides in different ways, so the same peptide may act as an acute blood-borne hormone, as a local growth factor, as a neurotransmitter, and as a fertility factor. The new biology suggests that gastrointestinal hormones should be conceived as intercellular messengers of general physiological impact rather than as local regulators of the upper digestive tract.

## I. INTRODUCTION

Gastrointestinal hormones are by all criteria ordinary hormones. Nevertheless, they have never been fully accepted in endocrinology. Endocrinology textbooks and general endocrinology meetings witness how gastrointestinal hormones often occupy only little room in today's concept of endocrinology. The restrained acceptance is a paradox in three ways. 1) Endocrinology was conceptually put on a firm scientific footing in the gut with the discovery of secretin (12, 13) and with the subsequent introduction of the word *hormone* (227). 2) The gut is the largest hormone-producing organ in the body, both in terms of number of endocrine cells and number of hormones (for comprehensive reviews, see Refs. 211 and 251). 3) The widespread expression of gastrointestinal hormone genes outside the gastrointestinal tract makes

the hormones multifunctional regulators of general physiological interest. Hence, gastrointestinal hormones may at the same time act as acute metabolic hormones, as neurotransmitters, as local growth factors, and as fertility factors. This widespread gene expression with tissue-specific prohormone processing combined with differential functions constitutes essential parts of what may be conceived as a new and fascinating biology, which should appeal also to general endocrinology and physiology.

The basic concept of endocrinology, blood-borne regulation by specific messenger molecules, was discovered in 1902 by the British physiologists William Maddox Bayliss and Ernest Henry Starling (12, 13). Following up on the observation of Pavlov and co-workers, that acidification of the upper intestine resulted in secretion of pancreatic juice (for review, see Ref. 178), Bayliss and Starling extracted from the duodenal mucosa a substance, which

injected into blood stimulated pancreatic bicarbonate secretion irrespective of whether the pancreas was innervated or not. They called the substance secretin, a somewhat unspecific designation. However, in 1905, Starling in his Croonian lecture proposed the word *hormone* as a general designation for blood-borne chemical messengers (227). That same year, John Sidney Edkins, also from University College in London, discovered another hormonal substance in extracts of the antral mucosa (67, 68). This substance stimulated gastric acid secretion. In view of its origin, Edkins called it "gastric secretin," but abbreviated it to gastrin. Hence, the two hormones first discovered in history were both gastrointestinal hormones. In the following decades, however, endocrinology as such blossomed by the discovery and isolation of steroid hormones from the adrenals, ovaries, and testes; larger protein hormones from the anterior lobe of the pituitary and oxytocin and vasopressin from the posterior pituitary lobe; and insulin from the pancreatic islets. In light of the immediate and often life-saving implications of these breakthroughs, the interest for secretin and gastrin faded to a degree that figuratively returned them to the darkness of the bowel. Subsequently, only a small priesthood of physiologists maintained an interest in the hormonal control of digestion. One of them was Andrew Ivy in Chicago, who in 1928 assisted by Eric Oldberg found evidence of a gallbladder-emptying hormone in extracts of the small intestine (115). He called the substance cholecystokinin (CCK). A stimulator of pancreatic enzyme secretion, termed pancreozymin, was subsequently discovered in the 1940s in small intestinal extracts by Harper and Raper in Newcastle (99). However, as shown in the 1960s by Erik Jorpes and Viktor Mutt in Stockholm, CCK and pancreozymin are one and the same substance (124, 165), for which now only the acronym CCK is used.

Secretin, gastrin, and CCK are the classical gut hormones. The troika was not only discovered first but structurally identified first (91, 93, 165–167). It was also by many believed that the endocrine regulation of digestion might be excreted only by these three hormones. A leading gastrointestinal physiologist in the 1960s and 1970s, Morton Grossman, even suggested that they acted via the same receptor (95). The trinity doctrine soon, however, turned out to be incorrect. There are many more gut hormones, and each has its own or even more receptors, although, vice versa, there are also examples showing that different gastrointestinal peptides may act on the same receptor. This complexity is also part of the new biology. However, so as not to lose sight in the avalanche of new information, the general and fundamental characteristics of the new biology are primarily exemplified with data for the classical gastrointestinal hormones. Moreover, only the molecular and cellular biology of the gastrointestinal hormones as such is reviewed. Neither transport mechanisms, receptors, nor signal transduction in target cells

are discussed here. In other words, this review attempts to present general principles governing structure and expression of gastrointestinal hormones. Readers interested in details about individual hormones and their effects should consult comprehensive multi-author volumes comprising the entire range of gastrointestinal endocrinology (211, 251) or the many volumes about individual gut hormones published in the 1990s. Before the characteristics and principles of the new biology of gastrointestinal hormones are presented, a summary of the old concept may be pertinent.

## II. THE CLASSIC CONCEPT

The old concept about gastrointestinal hormones, which prevailed unaffected through the century until the late 1970s, still dominates most general textbooks in physiology, biochemistry, and endocrinology. According to the classic biology, a gut hormone is a substance produced by one type of endocrine cell dispersed in a relatively well-defined region of the proximal gastrointestinal tract. From here it is released to blood by a specific stimulus to reach its target organ that subsequently elicits an acute response (secretion or muscle contraction).

In the 1960s, it was shown that gastrointestinal hormones could be peptides of some 20–30 amino acid residues. Hence, secretin, a 27-amino acid peptide, is released by gastric acid from S cells in the duodenum to stimulate bicarbonate secretion from exocrine pancreatic cells. Gastrin, a 17-amino acid peptide, is by protein-rich food in the stomach released from antral G cells to stimulate acid secretion from parietal cells in the fundic mucosa. And CCK, a 33-amino acid peptide, is by fat, protein, and acid released from small intestinal I cells to stimulate pancreatic enzyme secretion and gallbladder contraction. On the basis of simple physiological studies mainly in dogs, a number of additional gastrointestinal hormonal mechanisms were proposed in the first half of this century and as candidate hormones named according to function and origin (incretin, enterogastrone, duocrinin, antral chalone, villikin, and others). However, when the classic troika of hormones became available as pure peptides in the late 1960s and early 1970s, proper experimentation showed that several of the observed hormonal mechanisms could be explained either by interaction and/or additional effects of secretin, gastrin, and CCK or by newly identified gut hormones.

The new biology of gastrointestinal hormones maintains in accordance with the classic conceptions that the hormones are peptides, which are released to blood from cells in the gastrointestinal tract upon appropriate stimulation. However, the new biology contains a wealth of additional features revealed by modern molecular and cell biology. These features have been collected under five headings in this review.

For the sake of completeness, it should be added here that a number of monoamines (including histamine and dopamine) and various eicosanoids with hormonelike activities also are produced in the gastrointestinal tract. This review is, however, restricted to proper gut hormones, which are peptides. It should also be emphasized that the designations gastrointestinal hormones and gut hormones are used synonymously in this review.

### III. THE NEW CONCEPTS

#### A. Many Hormones and Their Families

Gastrointestinal endocrinology has since 1970, when it was confined to only three identified peptides (91, 166, 167), virtually exploded in number of regulatory gut peptides, i.e., hormones, peptide transmitters, and growth factors (Fig. 1). Not only have new peptides with all features of a hormone been found in gut extracts [gastric inhibitory polypeptide (GIP), Refs. 32, 34, 36; motilin, Refs. 31, 33, 35, 210; peptide tyrosine tyrosine (PYY), Refs. 236, 237; galanin, Refs. 72, 238; glucagon-like peptides (GLPs), Refs. 16, 109, 176, 177, 234, 239, 245], but also neuropeptides isolated from the central nervous system and hormones identified first in other endocrine organs have been found to be present in endocrine cells and/or neurons in the gastrointestinal tract [substance P, Refs. 43, 46, 71; the enkephalins, Refs. 73, 113; dynorphin, Ref. 224; neurotensin, Refs. 42, 98, 130; neuropeptide Y (NPY), Refs. 157, 232; the neurokinins, Ref. 55; pituitary adenylate cyclase-activating peptide, Refs. 158, 231; somatostatin, Refs. 8, 29, 47; pancreatic polypeptide (PP), Refs. 45, 129; and calcitonin gene-related peptide (CGRP), Refs. 2, 3, 164, 205, 241]. Moreover, potent regulatory peptides originally believed to be classical hormones but later shown to be widespread neurotransmitters have been isolated from gut extracts [vasoactive intestinal polypeptide (VIP), Refs. 137, 168, 207; peptide histidine isoleucine, Refs. 20, 114, 148, 237; and gastrin-releasing peptide (GRP), Refs. 153, 154, 156, 160]. Finally, a number of growth factors with hormonal effects have now been shown to be present in the gut: epidermal growth factor (EGF), originally isolated as a gut hormone; urogastrone, from urine independently of Cohen's EGF identification (49, 50), the isolation being monitored by its effect on gastric acid secretion by Harold Gregory (90); insulin-like growth factor I and II (30, 56, 209, 229); transforming growth factor (TGF)- $\alpha$  and - $\beta$  (14, 44, 134, 250); and amphiregulin (182).

The complexity is further increased by the fact that individual genes for gut regulatory peptides encode different peptides, which in a tissue- and cell-specific manner release a number of different bioactive peptides. Several principles for gene expression operate to provide such variety. Hence, alternative splicing of the calcitonin gene

transcript to express CGRP is not the only example (3). Also, the secretin gene is expressed in different molecular forms in the gut because of alternative splicing (84, 131).

Additional features contributing to the plurality are physiological studies which have indicated that there still are gut hormonal activities that are not easily explained by known peptides and, therefore, require identification of new hormones. Hence, as shown in Figure 1, 10 hormonal factors are still awaiting structural determination of both peptides and genes. Perhaps some of them can be partly explained by identified peptides. Hence, the incretin effect (135, 155) is probably exerted by a combination GIP (66) and GLP-I (54, 109) stimulations, whereas the entero-, vago-, and bulbogastrone effects may be explained by various combinations of somatostatin, GIP, EGF, and TGF- $\alpha$ . However, villikin, duo- and enterocrinins, and the recently suggested gastrocalsin (181) still await a structural identification of new substances, most probably peptides.

It may be difficult to overlook the multiplicity of the gut peptide systems. The structural identifications, however, have simplified the matter by showing striking homologies between groups of peptides. Consequently, one-half of the hormones can be classified in families based on homology. Table 1 shows the major gastro-entero-pancreatic hormone families. The expression of several peptide genes both in the gut and the pancreas reflects that the pancreas is of intestinal origin, both in onto- and phylogenetic terms.

The nature of the homology varies from family to family. It may be an overall similarity in the primary structure as illustrated by the PP-fold family, which comprises PP, PYY, and NPY (Table 1). The family members display similarities varying between 45 and 70% (Fig. 2). The extensive similarity of the primary structures is coupled to an almost identical and stable tertiary structure because the homology explicitly comprise residues, which are important for stabilization of the three-dimensional PP-fold structure (88). The PP-fold motif consists of a polyproline-like helix (residues 1–8) and an amphiphilic  $\alpha$ -helix (residues 15–30). The two helices are joined by a type I  $\beta$ -turn (residues 9–12) and held in the folded configuration by hydrophobic interdigitations between side chains of the  $\alpha$ -helix residues and the  $\text{NH}_2$ -terminal proline residues (Fig. 2). Not only are the bioactive 36-amino acid peptides in the family highly homologous, the cDNA-deduced prepropeptides also display remarkable similarities in their organization (21, 147, 157).

Another type of homology is that of the gastrin family (Table 1 and Fig. 3). The family comprises, in addition to the mammalian hormones, gastrin and CCK, also the protochordate neuropeptide cionin (120), and the frog skin peptide cerulein (4). The decisive homology of this family is concentrated in and around the precisely defined active site, the common COOH-terminal tetrapeptide am-

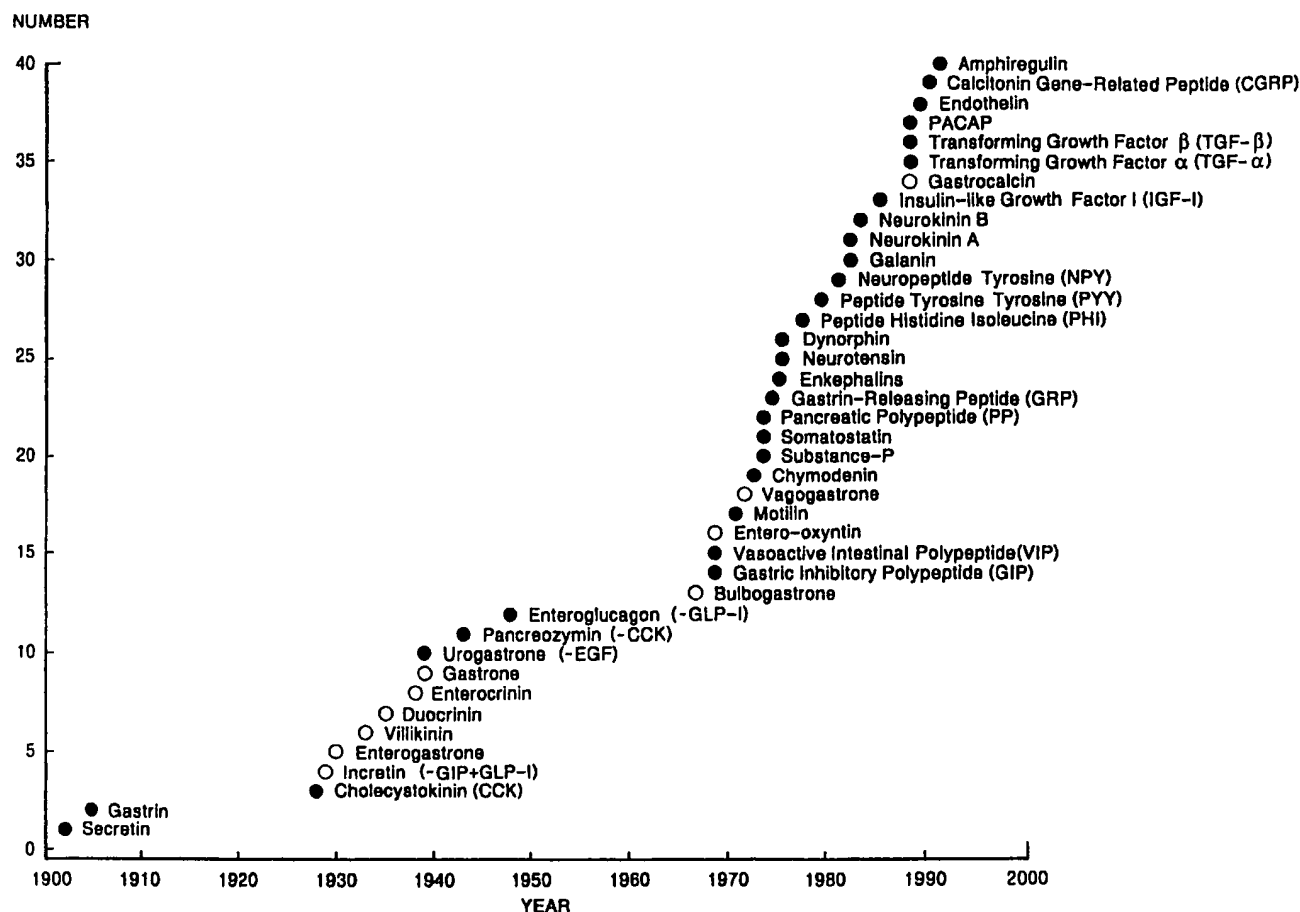


FIG. 1. Discovery and identification of regulatory peptides in gastrointestinal tract. Peptides may act as hormones, neurotransmitters, and growth factors. Sometimes 1 peptide acts in 2 or all of the 3 roles. Discovery is indicated by year of first report. Solid circles indicate structural identification, and open circles indicate hormonal activities, which still require identification of responsible hormone(s). Some of structurally unidentified hormonal activities can be partly explained by activity of later identified hormones; for instance, incretin activity is partly due to gastric inhibitory polypeptide (GIP) and glucagon-like peptide I (GLP-I) activities. Commonly used acronyms are indicated in brackets after full name, except for PACAP, which is an acronym for pituitary adenylate cyclase-activating peptide.

ide -Trp-Met-Asp-Phe-NH<sub>2</sub> (the box in Fig. 3). Any modification of this site grossly reduces or abolishes the receptor binding and consequently the biological effects of the hormones (162). Comparison of propeptide and gene structures reveals a complex pattern with an overall similarity in the organization of progastrin, proCCK, and procinonin as well as their genes (22, 57, 58, 161, 203, 256, 257, 259), but with only little similarity in the amino acid residues and DNA sequences outside the sequence corresponding to the common active site and its COOH-terminal flanking peptide. Hence, this family is in contrast to the PP-fold family and the secretin family primarily defined by the conserved active site sequence and by the neighboring O-sulfated tyrosyl residues (Fig. 3).

The frequent occurrence of homology among gastrointestinal hormones, peptide neurotransmitters in the gut, and intestinal growth factors is not a feature specific for

regulatory peptides in the gut. On the contrary, it is common among all kinds of regulatory peptides, enzymes, and other proteins in the organism (for reviews, see Refs. 1, 64, 65, 118, 260). Each family is assumed to reflect the phylogenetic evolution by duplication and subsequent mutations of an ancestral gene. It is, however, possible that the homology not only reflects divergent evolution. The occurrence of homologous peptides in submammalian species may also demonstrate the existence of several related genes, of which some do not evolve into genes of mammalian species or of other vertebrates.

The question of phylogenetic origin has recently been examined in detail for the gastrin family by Johnsen (118). His study tested an immunochemically based hypothesis suggesting that CCK and gastrin originate from a single common ancestral gene, which during evolution duplicated into separate CCK and gastrin genes

TABLE 1. *Gastroenteropancreatic peptide families*

<b>Secretin family</b>	<b>Gastrin family</b>
Secretin	Gastrin
* { Glucagon and glucagon-like peptides	Cholecystokinin
Gastric inhibitory polypeptide	Cerulein†
* { Vasoactive intestinal polypeptide and Peptide histidine isoleucine	Cionin†
Growth hormone releasing hormone	<b>PP-fold family</b>
Pituitary adenylyl cyclase-activating peptide	Pancreatic polypeptide
	Peptide YY
	Neuropeptide Y
<b>Insulin family</b>	<b>Tachykinin family</b>
Insulin	Substance P
Insulin-like growth factor I	Neurokinin A
Insulin-like growth factor II	Neurokinin B
Relaxin	
<b>EGF family</b>	<b>Somatostatin family</b>
Epidermal growth factor	Somatostatin
Transforming growth factor- $\alpha$	Corticotropin
Amphiregulin	

\* Peptides encoded by one gene. † Not present in mammals.

at the level of reptiles (140). Identification of peptides from brain and gut tissues of species representing the entire animal kingdom (including invertebrates) showed, however, the following: so far it has not been possible in invertebrates to identify true CCK-/gastrin-like peptides

having an intact COOH-terminal tetrapeptide amide (Fig. 3), although invertebrate neurons may express a family of less related -Asp-Phe-NH<sub>2</sub> peptides (122). Possibly, however, the gastrin family may represent a subclass of a larger DFamide family. The earliest occurrence of true gastrin/CCK peptides in evolution is apparently cionin as expressed in protochordate neurons (120; Fig. 3). Procionin, the cionin gene, and its expression pattern resemble mammalian CCK rather than gastrin (161). So far, therefore, the gastrin family appears to originate in protochordates with expression of the CCK-like cionin gene at the evolutionary level, where vertebrates branch off from invertebrates ~500 million years ago or earlier. In cartilaginous fish or elasmobranchs (the earliest animals to secrete gastric acid), the CCK-like gene has duplicated to express two very similar peptides of which one is likely to regulate gastric acid secretion (119). Two such CCK-like peptides are also expressed in the gut of bony fish, amphibians, reptiles, and birds (18, 121). Only mammals express gastrins with a structure that differs grossly from CCK outside the active site sequence (22, 118, 259).

The phylogenetic story of the gastrin family shows that gastrointestinal hormones indeed are very old. So far, the data also support the idea that each gut peptide family has evolved from a single ancestor. An associated trait is that gastrointestinal hormones, at least in the gastrin family, to a large degree have preserved their tissue-specific

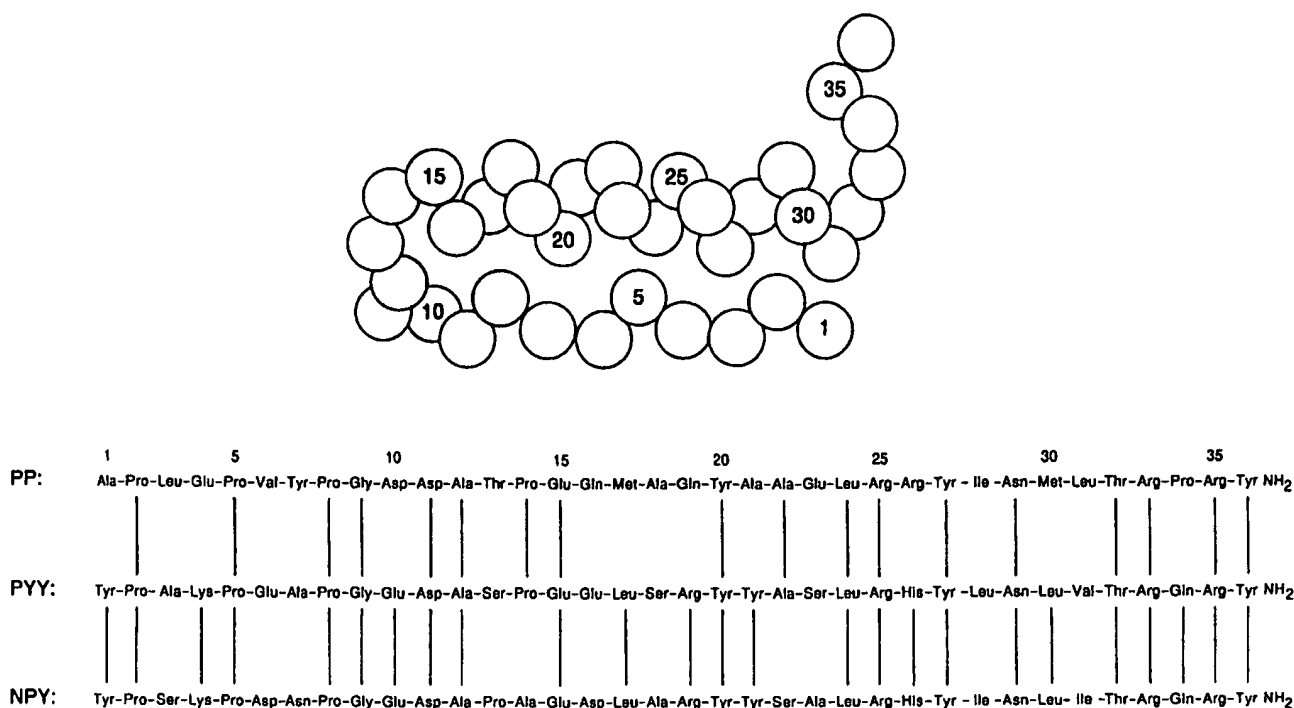


FIG. 2. Structural homology of members of pancreatic polypeptide (PP)-fold family: PP, peptide tyrosine tyrosine (PYY), and neuropeptide tyrosine (NPY). *Top*: PP-fold configuration (tertiary structure) of the three 36-amino acid peptides. *Bottom*: amino acid sequences (primary structure) of the three porcine peptides.

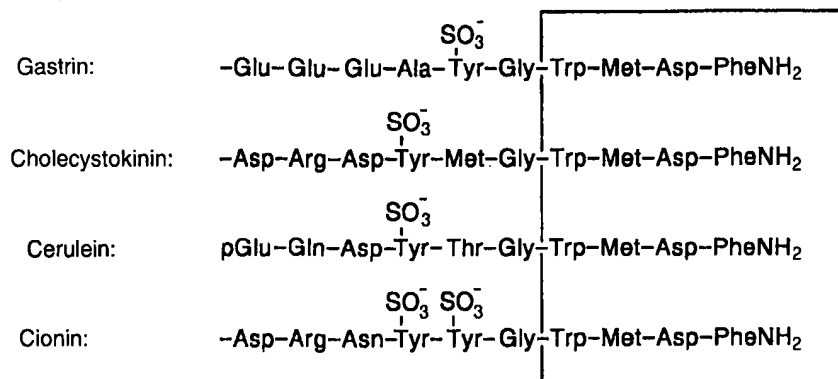


FIG. 3. Structural homology of members of gastrin family: gastrin, cholecystokinin (CCK), cerulein, and cionin. The common COOH-terminal tetrapeptide amide in box constitutes minimal structure necessary for bioactivity. Note position of characteristic *O*-sulfated tyrosine in the 4 peptides. Shown poly-Glu sequence in gastrin decapeptide is that of human, pig, rat, and mouse.

sites of expression during evolution, both primary and secondary sites (206). Accordingly, both the structural and cell-specific evolutionary conservation emphasize the general biological significance of gut hormones.

## B. Multiple Phenotypes of a Hormone Gene

A quarter of a century ago, gastrointestinal endocrinology was, as mentioned, believed to comprise three different peptides (secretin, gastrin, and CCK). At that time it was also believed that one gene encoded one hormonal molecule, in accordance with what was learned about the master hormone, insulin. In other words, one gene equals one hormonal peptide. The complexity of gastrointestinal endocrinology first grew in a straightforward manner with the discovery and recognition of new hormones (Fig. 1). However, the synchronous rapid development in peptide chemistry and molecular biology soon added new and more intricate dimensions to the complexity. The "one gene, one hormonal peptide" dogma turned out to be too simplistic for many gut hormones. Instead, and concurrently with the elucidation of eukaryotic gene structures and improved understanding of the mechanisms governing gene expression cascades, it became obvious that hormone genes often express several different bioactive peptides. Exactly which peptides appear to be regulated in a cell-specific manner? Today we know three different ways in which a gastrointestinal hormone gene can express different bioactive peptides (Fig. 4).

### 1. Alternative splicing of transcripts

Existence of different molecular forms of a gut hormone or of an intestinal peptide neurotransmitter is quite common. It is important to emphasize, however, that the concept of molecular heterogeneity only refers to the existence of more than one bioactive form. The coexistence of more or less inactive prohormones and processing intermediates and/or degradation fragments with a single proper hormone, as well known for insulin, is trivial. As

described later for gastrin, true molecular heterogeneity most often reflects an elaborate posttranslational phase of the expression cascade, which results in cellular synthesis and release of several different forms of a hormone. Another and apparently less common way in which a gene can express different molecular forms is by alternative splicing of RNA transcripts.

Alternative splicing, as a mechanism by which peptide hormone complexity increases, was discovered 15 years ago, when Amara and co-workers (2, 3, 205) showed that calcitonin gene transcription generates different mRNA encoding either calcitonin peptides or CGRP. It is now also well established that CGRP are abundantly expressed in intestinal neurons throughout the digestive tract (164, 241; for review, see Ref. 110). Hence, alternative splicing does play a significant role in the control of digestive functions.

The calcitonin gene is, however, not the only gene encoding a gut peptide, which expresses itself by alternative splicing. The alternative splicing of a tachykinin gene (171) and also one of the peptides encoded by the secretin gene is a result of alternative splicing of the primary transcripts (84, 131).

For many years, secretin was believed to exist only as a carboxyamidated peptide of 27 amino acid residues (166, 167). However, in the mid 1980s, two additional secretins with full bioactivity were identified in porcine gut extracts. One was the immediate precursor of amidated secretin-27, i.e., glycine-extended secretin-28 (41), and the other was secretin-30 extended by a Lys-Arg sequence (83). In contrast to members of the gastrin, tachykinin, and PP-fold families (Table 1 and Figs. 2 and 3), COOH-terminal extensions of the amidated end products of secretin, VIP, and other members of the secretin family do not eliminate or reduce the bioactivity, because the activity resides mainly in the NH<sub>2</sub> terminus of the peptides.

The existence of glycine- and glycyl-lysyl-arginine-extended forms of secretin and VIP is not surprising. They are in fact to be expected from what is known about the biosynthesis of carboxyamidated peptides. Unexpected

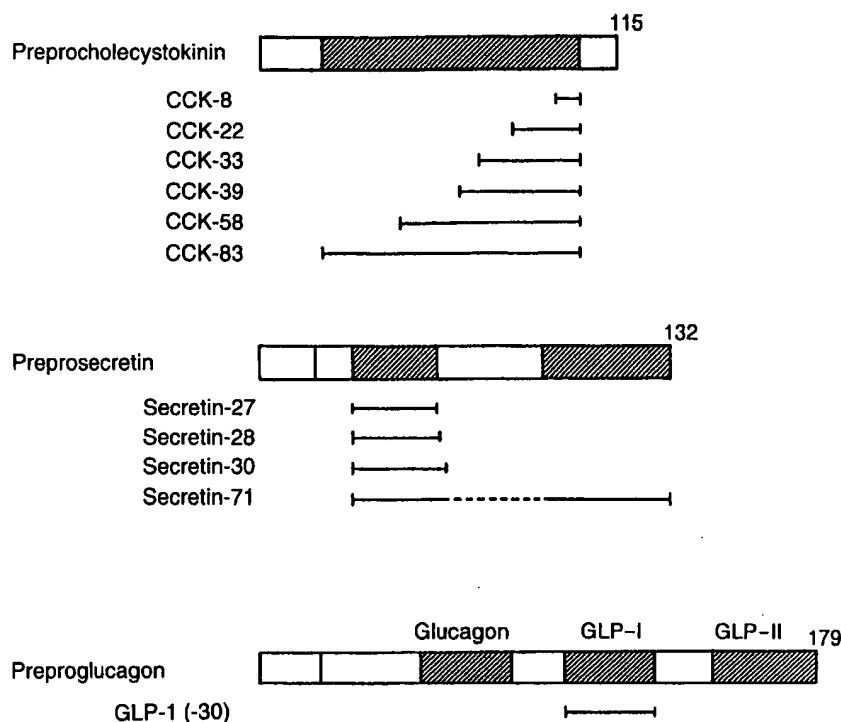


FIG. 4. Multiple phenotypes (molecular heterogeneity) of 3 gut hormone genes. The cholecystokinin (CCK) gene encodes a prepropeptide, which through differentiated endoproteolytic cleavages is processed to 6 CCK peptides varying in length from 83 to 8 amino acid residues. The six peptides have the same COOH-terminal bioactive octapeptide sequence (see also Fig. 3). The secretin gene encodes a prepropeptide that through endoproteolytic cleavages and variable COOH-terminal trimming is processed to 3 bioactive secretin peptides of almost similar size (secretin-27, -28, and -30). In addition, bioactive secretin-71 is produced by splicing out RNA encoding midsequence of preprosecretin (i.e., broken line of secretin-71). The glucagon gene encodes a prepropeptide that through cell-specific endoproteolytic cleavages is processed to either genuine pancreatic glucagon (in pancreatic  $\alpha$ -cells) or to glucagon-like peptides I and II (GLP-I, GLP-II; in intestinal L cells).

was, however, the discovery of secretin-71 (84), which contains the sequence of nonamidated secretin-27 NH<sub>2</sub> terminally, followed by a Gly-Lys-Arg extension and a further COOH-terminal extension of 41 amino acid residues (Fig. 4). With the exception of an arginine residue, which follows the Gly-Lys-Arg sequence directly, the COOH-terminal sequence of secretin-71 is identical to the COOH-terminal 40 amino acid residue fragment of porcine preprosecretin (Fig. 4). Thus what corresponds to secretin RNA encoding a 32-amino acid sequence has been spliced out from the primary secretin gene transcript. For reasons mentioned above, secretin-71 has full secretin bioactivity. It remains to be settled how large a fraction secretin-71 constitutes of the circulating secretins. The large molecular size suggests a slow metabolic clearance from blood. Secretin-71 should therefore accumulate in plasma and constitute a significant fraction. It also remains to be settled how common alternative splicing of the primary secretin gene and other gastrointestinal hormone gene transcripts are among vertebrates.

## 2. Multiple products of prohormones with one active sequence

Members of the somatostatin and gastrin families represent peptide systems in which the gene encodes only one prohormone and in which only one prohormone with one active site is expressed, but nevertheless processed in a way so that a number of molecular forms of different

lengths with the same bioactive COOH terminus are synthesized (Table 2). Although the different bioactive products of the same precursor are bound to the same receptor, their varying metabolic clearances from plasma affect their hormonal significance considerably. Hence, it matters indeed whether intestinal proCCK is processed mainly to CCK-58 or to CCK-8 or whether prosomatostatin is processed to somatostatin-28 or -14.

So far, the processing of progastrin in antral G cells has been examined particularly thoroughly. It will therefore be used to illustrate the second way in which one gastrointestinal hormone gene can encode different bioactive peptides.

In vertebrates, antral G cells synthesize by far most of the gastrin of the total organism (Table 3). Through a combination of knowledge about progastrin structure (22, 80, 82, 85, 123, 149, 206, 259, see also Fig. 5), classic bio-

TABLE 2. Molecular forms in primary sites/cells of synthesis

Hormone	Cell	Bioactive Prohormone Products
Secretin	S cells	Secretin-71, -30, -28, and -27
Gastrin	G cells	Gastrin-71, -34, -17, and -6
Cholecystokinin	I cells	CCK-83, -58, -39, -33, -22, -8, and -5

Secretin-71 is synthesized by alternative splicing of secretin gene transcript. Gastrins are both O-sulfated and nonsulfated (except gastrin-6, which is fully sulfated). Cholecystokinins (CCK) are completely O-sulfated (except CCK-5 without a tyrosyl residue).

TABLE 3. *Expression of progastrin and its products in mammalian tissue*

Tissue	Total Translation Product, pmol/g tissue	Precursor Percentage
Gastrointestinal tract		
Antral mucosa	10,000	5
Duodenal mucosa	400	20
Jejunal mucosa	40	30
Ileal mucosa	20	85
Colonic mucosa	0.2	100
Neuroendocrine tissue		
Cerebellum	5	20
Vagal nerve	8	10
Adenohypophysis	200	98
Neurohypophysis	30	5
Adrenal medulla	2	100
Pancreas	2	95
Genital tract		
Ovaries	0.5	100
Testicles	6	100
Spermatozoa	2	55
Respiratory tract		
Bronchial mucosa	0.3	100

Orders of magnitude are based on examination of different mammalian species in the laboratory of the author (10, 26, 28, 79, 89, 117, 150, 151, 185, 186, 189, 191, 193, 196, 197, 199, 221, 222, 244).

synthesis studies (28, 62, 106, 230, 246, 247), identification of progastrin products in antral extracts (91, 93, 94, 192, 194), and modern cell biology (38, 40), the biosynthetic pathway and the way in which G cells provide different gastrins are now well established (Fig. 6).

After translation of gastrin mRNA in the rough endoplasmic reticulum and cotranslational removal of the NH<sub>2</sub>-terminal signal peptide from preprogastrin, intact progastrin is transported to the Golgi apparatus. In the *trans*-Golgi network, the first posttranslational modifications occur. These are *O*-sulfation of the tyrosyl-66 residue neighboring the active site and the first of the endoproteolytic prohormone convertase cleavages. From the *trans*-Golgi network, vesicles carry the processing intermediates of progastrin toward the basal part of the G cells, where the gastrin peptides are stored in characteristic secretory granules (61, 76, 97, 141, 163, 183). We assume that the endoproteolytic prohormone convertase cleavages at two monobasic and three dibasic processing sites (59, 152, 212, 216, 217, 228) and exoproteolytic carboxypeptidase E trimmings (77, 78) as well as the subsequent glutamyl cyclization (75), corresponding to the NH<sub>2</sub> terminal of gastrin-34 and gastrin-17, continue during the transport from the Golgi to the early secretory granules. The last and decisive processing step in the synthesis of gastrin then occurs during storage and maturation in the secretory granules. The secretory granules contain the amidation enzyme complex (peptidylglycine  $\alpha$ -amidating monooxygenase or PAM, Refs. 59, 60, 69, 106), which removes glyoxylate from the immediate precursors, the gly-

cine-extended gastrins, to complete the synthesis of bioactive  $\alpha$ -carboxyamided peptides (Fig. 6). Amidation of gastrin is a crucial all-or-none activation process, which is carefully controlled (60, 102, 106, 107, 117). Activation of the enzymatic amidation process requires copper, oxygen, ascorbic acid, and a pH of  $\sim 5$  (70, 102, 107; for reviews, see Refs. 69 and 103). Carboxyamidation of peptides has now been shown to require two sequentially acting enzymes: a copper and ascorbate-dependent peptidylglycine  $\alpha$ -hydroxylating monooxygenase, derived from the NH<sub>2</sub>-terminal part of the PAM precursor, and a separate peptidyl- $\alpha$ -hydroxyglycine  $\alpha$ -amidating lyase, derived from the remaining intragranular region of the PAM precursor (127, 179, 233, 235). Partial phosphorylation of serine in the COOH-terminal flanking fragment of progastrin also occurs (62, 246, 247). The significance of the phosphorylation is not yet known and the kinase is unknown, but it is possible that phosphorylation may decrease the intracellular processing period in G cells (247, 248).

As a result of the elaborate biosynthetic pathway, the normal antral G cells in humans release a heterogeneous mixture of progastrin products from the mature secretory granules. A small percentage are nonamidated precursors, mainly glycine-extended gastrins. However, in humans, more than 90% are  $\alpha$ -amidated bioactive gastrins of which the longest molecular form is gastrin-71 (194). Of the amidated gastrins, 90% are gastrin-17, 5% are gastrin-34, and the rest is a mixture of gastrin-71, -52, -14, and short COOH-terminal hepta- and hexapeptide amide fragments (92, 104, 117, 151, 192, 194, 196, 199). Approximately one-half of the amidated gastrins are tyrosyl sulfated (5, 6, 28, 94, 106). Because of major differences in metabolic clearance rates, the distribution of gastrins in peripheral plasma changes so that larger gastrins with their long half-lives predominate over gastrin-17 and shorter gastrins (117).

Increased gastrin synthesis changes the molecular pattern further. Abnormally increased antral synthesis occurs by achlorhydria, as seen in pernicious anemia. In antrum-sparing pernicious anemia, the translational activity of gastrin mRNA in the G cells seems to be so high that the enzymes responsible for the processing of progastrin cannot keep up with the maturation, i.e., the carboxyamidation process (117). Consequently, G cells release more unprocessed and incompletely processed nonamidated progastrin products when the synthesis is increased. Also, the carboxyamided gastrins are less sulfated (7), and the NH<sub>2</sub> terminus of progastrin is less cleaved (117). Precursors, processing intermediates, and long-chained carboxyamided gastrins, such as gastrin-71, gastrin-52, and gastrin-34, are, as mentioned, cleared at a relatively slow rate from the circulation and therefore accumulate in plasma when synthesis and release are increased. Therefore, assays that measure progastrin and its products irrespective of the degree of processing provide a better mea-



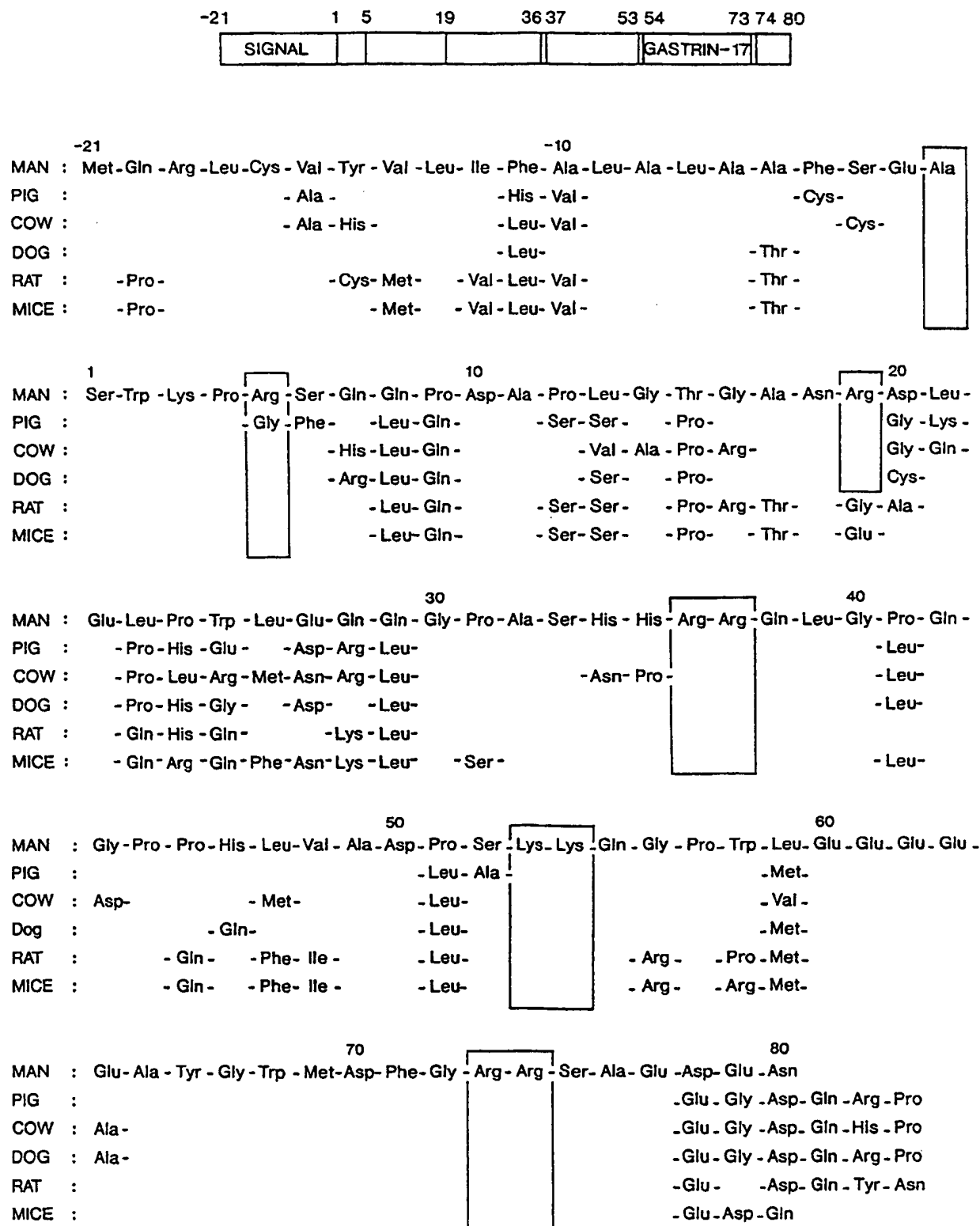


FIG. 5. cDNA-deduced amino acid sequences of preprogastrin from 6 mammalian species. Boxes indicate endoproteolytic cleavage points of mono- and dibasic processing sites. Note preserved primary structures around bioactive site (sequence 68–71, see also Fig. 3) and around endoproteolytic processing sites.

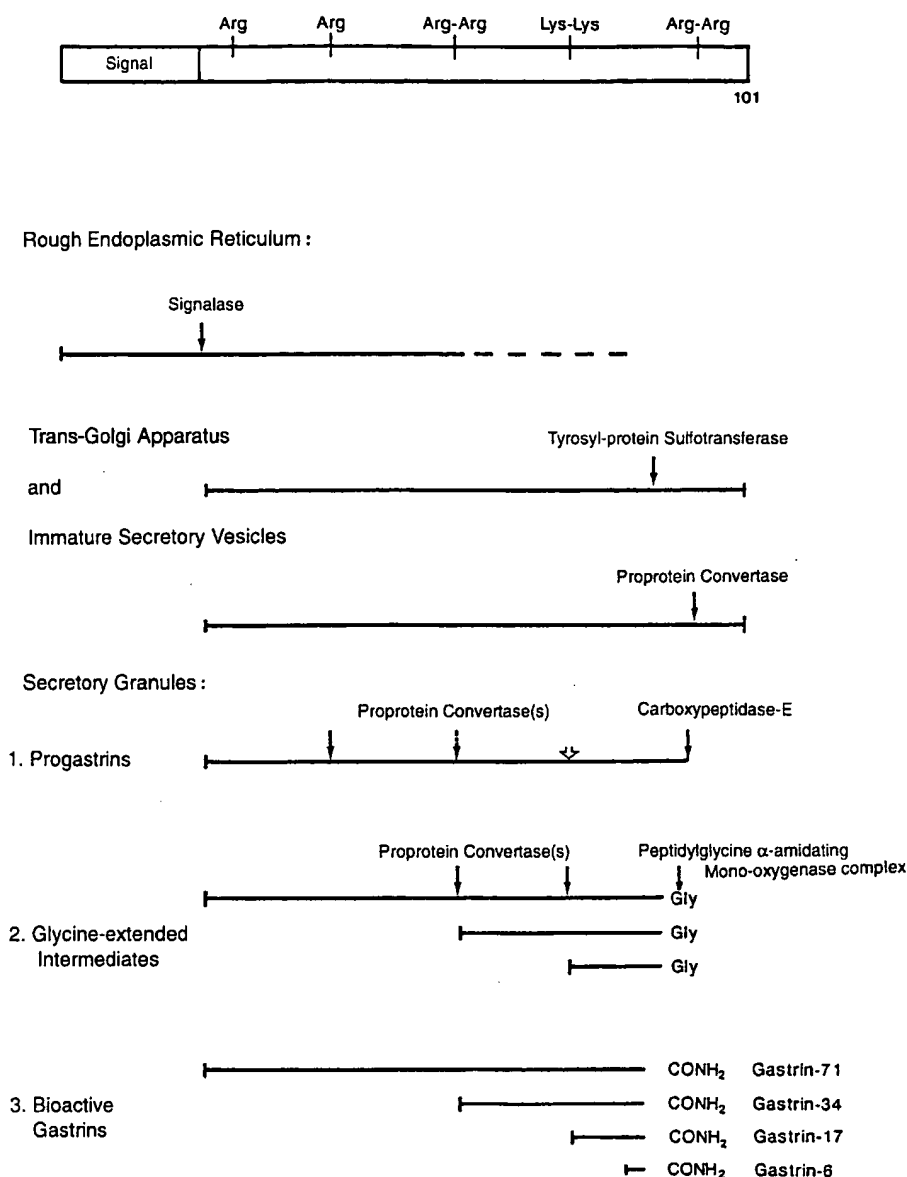


FIG. 6. Diagrammatic illustration of co- and posttranslational processing of preprogastrin in human antral G cells. Note that processing comprises both endoproteolytic cleavages (by signalase and proprotein convertases), exoproteolytic trimming (by carboxypeptidase E), and amino acid derivatizations (by tyrosyl-protein sulfotransferase and by peptidyl-glycine  $\alpha$ -amidating monooxygenase complex).

sure of increased gastrin synthesis than conventional assays, which recognize only the fully processed amidated gastrins (11).

### 3. Differential processing of prohormones containing two or more active sequences

A third way in which expression of one gene can produce different bioactive peptides occurs when the gene encodes a propeptide containing different but often homologous peptide hormones or neuropeptides (Fig. 4). Gut neuroendocrinology comprises many examples of such genes of which the opioid-peptide genes (51, 96, 126, 169, 175), some of the tachykinin genes (133, 170, 171),

the VIP gene (20, 114, 148, 258), the galanin gene (72, 204, 249), and the glucagon gene (15, 16, 101, 255) amply illustrate the phenomenon. Some of the genes not only encode a peptide precursor containing different bioactive peptides, which is then subjected to tissue-specific post-translational processing, but the primary transcripts of these gene(s) may also undergo tissue-specific alternative splicing (171).

Preproglucagon is an example of a poly-protein precursor, which in mammals contains three similar but still different peptide sequences (Fig. 4). In the  $\alpha$ -cells of the pancreatic islets, proglucagon is processed to release the well-known pancreatic glucagon, whereas the COOH-terminal part of proglucagon remains silent in the sense that

neither GLP-I nor GLP-II is synthesized (177, 245). The L cells of the gut express proglucagon even more abundantly than the pancreatic  $\alpha$ -cells but process the precursor in a different way to release mainly GLP-I (109, 176, 177; for review, see Ref. 108). Although glucagon and GLP-I are highly homologous peptides and both are glucoregulatory, they have separate activities and, accordingly, separate receptors (17, 116, 240, 243). Hence, they are truly different hormones. Although the human gastrointestinal tract produces only GLP-I and not glucagon (108), the stomach of mammals like dog, cat, and rat contain so-called A-like cells, which produce glucagon in the same way as the pancreatic  $\alpha$ -cells (63, 139). Hence, in some animals, proglucagon delivers two different gut hormones.

Proglucagon tells another story of general interest. Deduction of its structure from cloned cDNA provided, in the case of preproglucagon, the first evidence or suggestion of separate bioactive peptide moieties from the same precursor due to the striking homologies between the sequences 33–61 (pancreatic glucagon), 72–107 (the original GLP-I), and 126–158 (GLP-II) (15, 16). Physiological studies showed, however, that the first deduced and proposed GLP-I (proglucagon 72–107), which is situated between two dibasic sites in the precursor, is a poorly active peptide (108, 109). Instead, a truncated form of the original GLP-I, which corresponds to the proglucagon sequence 78–107, turned out to be a highly potent glucoregulatory peptide (109). Moreover, it is the shorter fragment 78–107 that is synthesized and secreted in the gut (109, 176) and that therefore is the genuine GLP-I hormone. The lesson of this story is, consequently, that bioactive peptide structures cannot be predicted from cDNA and precursor sequences. It requires also precise identification of the released peptides accompanied by proper physiological studies of their activities.

### C. Widespread Gene Expression

The term *gene expression* is widely used. Unfortunately, however, it is also broad and is often used in an unspecific manner. Sometimes it refers only to demonstration of mRNA molecules, irrespective of whether the message is translated or not. On other occasions, it refers to synthesis of the primary translational product, irrespective of whether the protein product is processed to a functionally active form. Finally, it may be used in a more phenotypic sense to indicate the presence of the protein or peptide in its bioactive form, i.e., the phenotypic end point of expression.

For gastrointestinal hormones and other regulatory peptides, the expression cascade is generally highly elaborate and involves multiple enzymatic steps. Each of these steps may control whether the initial transcription pro-

TABLE 4. *Expression of procholecystokinin and its products in mammalian tissue*

Tissue	Total Translation Product, pmol/g tissue	Precursor Percentage
Gastrointestinal tract		
Duodenal mucosa	200	5
Jejunum mucosa	250	20
Ileal mucosa	20	50
Colonic mucosa	5	50
Neuroendocrine tissue		
Adenohypophysis	25	100
Neurohypophysis	20	10
Thyroid gland	2	20
Adrenal medulla	1	50
Genital tract		
Testicles	5	80
Spermatozoa*		
Central nervous system		
Cerebral cortex	400	2
Hippocampus	350	2
Hypothalamus	200	2
Cerebellum	2	80

Orders of magnitude are based on examination of different mammalian species in the laboratory of the author (79, 142, 150, 159, 180, 184, 186, 187, 193, 196, 198). \* Cholecystokinin peptides are present in spermatozoa of nonhuman mammals. The concentration, however, has not been quantitated.

cess in the end results in a bioactive product. It is in fact not unusual to see gene transcription without the encoded bioactive end product. Accordingly, reports on lack of parallelism and synchrony between the occurrence of mRNA, propeptide, and the mature bioactive peptide are steadily increasing (for review, see Ref. 188; see also Tables 3–5). Hence, in discussions of the often widespread expression of the genes for peptide hormones and other regulatory peptides in “new” sites in the body, it is necessary to specify the sense in which the term *expression* is meant. Especially in a physiological context, it becomes important to know whether transcription results in a bioactive product. Otherwise, what, for instance, is the physiological significance of the abundant but untranslated secretin mRNA in the colorectal mucosa (131; Table 5)? In other words, it is not possible to conclude that mRNA occurrence means synthesis of functionally active peptides.

All gut hormones are widely expressed in tissues outside the gastrointestinal tract, also phenotypically in terms of bioactive peptides. For some, the extraintestinal expression is confined mainly to neuroendocrine cells, especially to neurons in the central and peripheral nervous systems. However, several gastrointestinal hormones are expressed also in other cell types and tissues (Tables 3–5). The literature on extraintestinal expression of gut hormones is by now overwhelming. Therefore, the phenomenon will be illustrated with two examples only, namely, gastrin and CCK. Also, only sites of expression will be

TABLE 5. *Expression of secretin peptide and mRNA in mammalian tissue*

Tissue	Secretin Peptide, pmol/g tissue	mRNA (Relative Abundance)
Gastrointestinal tract		
Duodenal mucosa	40	+++
Jejunum mucosa	15	+++
Ileal mucosa	15	++++
Colonic mucosa	0	+
Neuroendocrine tissue		
Cerebral cortex	2	(+)
Striatum	4	(+)
Hippocampus	4	(+)
Hypothalamus	10	+
Midbrain	5	(+)
Medulla oblongata	3	++
Pineal gland	30	-
Pituitary gland	30	++
Pancreas	15	++
Urogenital tract		
Kidney	0	(+)
Testicles	0	(+)
Respiratory tract		
Bronchial mucosa	0	(+)

Orders of magnitude are based on examination of different mammalian species (131, 146).

mentioned where proper gastrin or CCK peptides have been demonstrated. The gastrin gene expression occurs widely and promiscuously in many different cell types, whereas CCK exemplifies extraintestinal expression restricted to neuroendocrine cells.

The gastrin gene is as mentioned expressed at the peptide level in several other cell types than the antroduodenal G cells. Quantitatively, these other cells produce only little of the gastrin released to blood in normal organisms. This is partly because the extra-antral secretion seems to serve local purposes rather than a general endocrine purpose, and partly because the biosynthetic processing often is cell specific, i.e., so different from that of the antral G cells that bioactive amidated gastrin may not even be synthesized. So far, we have encountered expression of progastrin and its products outside antroduodenal mucosa in the distal small intestine, perhaps originating from the so-called TG cells (37, 79, 141); in unidentified cells in the colorectal mucosa (150, 151); in endocrine cells in the fetal and neonatal pancreas (10, 26, 144); in pituitary corticotrophs and melanotrophs (143, 185, 197); in oxytocinergic hypothalamopituitary neurons (184, 186, 193); in a few cerebellar (189) and vagal neurons (244); in the adrenal medulla of some species (unpublished results); in the bronchial mucosa (191); in postmenopausal ovaria (222); and in human spermatogenic cells (208). As shown in Table 3, the concentrations and presumably also the synthesis in the extra-antral tissues are in adult mammals far below that of the antral "main factory."

The precise function of gastrin synthesized outside the antroduodenal mucosa is unknown, but several sug-

gestions can be offered. First, an obvious possibility is local paracrine or autocrine regulation of growth. Second, it is possible that the low concentration of peptides is without significant function in the adult but is a relic of a more comprehensive fetal synthesis meant for local stimulation of growth (10, 150). A third possibility is that the low cellular concentration is due to constitutive rather than regulated secretion (128). With constitutive secretion, there is no storage of peptides in the cells in spite of a considerable release per time unit.

Recent studies of gastrin-deficient "knock-out" mice have so far shown that antral gastrin is of utmost significance for development of the stomach and gastric acid secretion (81). However, clues to the significance of extra-antral gastrin are still lacking and awaiting further studies of double and triple knock-out mice.

The CCK gene is expressed at the peptide level in several other cell types than the small intestinal I cells. However, in contrast to the homologous gastrin gene, CCK gene expression is confined to neuroendocrine cells (Table 4) of which CCK neurons in the brain predominate. Neurons in all regions of the central nervous system synthesize carboxyamidated CCK peptides, although cerebellar neurons only in the fetal state (142, 159, 184, 198). The highest expression occurs in neocortical regions, which explains why CCK is the most abundant peptide system in the mammalian brain (52, 184). Cholecystokinin peptides are also widely expressed in peripheral neurons, primarily in the intestinal tract, but also in the genitourinary tract and elsewhere (142). Low-level expression has been found in pituitary corticotrophs (186, 187), in thyroid C cells (195), in the adrenal medulla (9), in the bronchial mucosa (86), and in spermatogenic cells of certain species (180).

Although neuronal CCK peptides act as potent transmitters in central and peripheral synapses, the roles, if any, of CCK peptides in pituitary, thyroid, and adrenal endocrine cells are unknown. Neuronal CCK peptides seem to play decisive roles in the development of anxiety and panic disorders in humans and other mammals (23–25, 190) as well as some role in satiety disorders (87, 218). The exact molecular and neuronal mechanisms in these disturbances remain to be clarified.

A striking difference in the cellular expression of the otherwise homologous gastrin and CCK genes (57, 256) is the high degree of promiscuity in the expression of the gastrin gene. Cholecystokinin peptides are, with the exception of germ cells (180), apparently never synthesized outside endocrine cells or neurons. Also, when it comes to neoplasia, benign as well as malignant, CCK peptides only occur in tumors of neuroendocrine origin (for review, see Ref. 200). In contrast, gastrin peptides are synthesized in many other types of cells than neuroendocrine, and this feature becomes further prominent in tumors (200). Hence, gastrins have been found not only

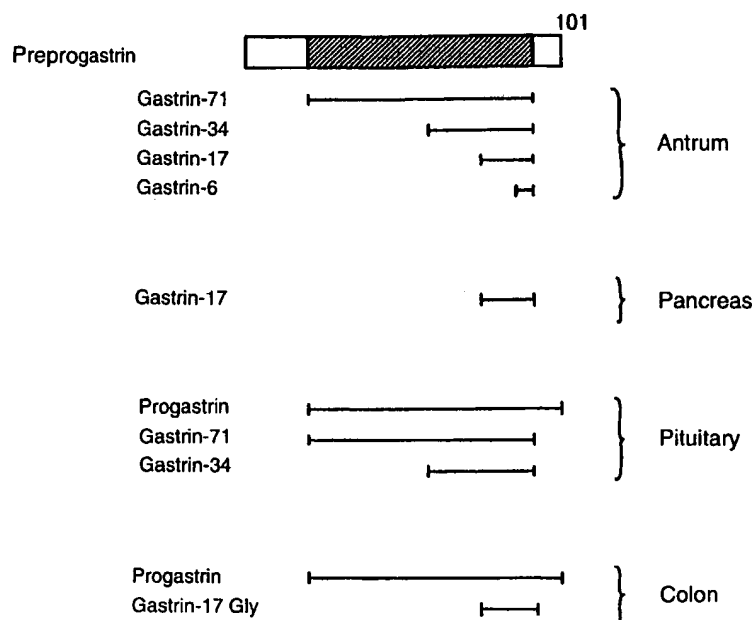


FIG. 7. Schematic illustration of cell-specific processing of preprogastrin in antral G cells (see also Fig. 6), G cells in fetal and neonatal pancreas, in pituitary corticotrophic cells, and in unidentified cells in colorectal mucosa.

in the relatively rare neuroendocrine tumors but also in significant amounts in common carcinomas such as bronchogenic, colorectal, gastric, pancreatic, and ovarian cancers (48, 89, 173, 191, 221–223; for review, see Ref. 200). Interestingly, local expression of the gastrin receptor seems to accompany the widespread expression of gastrin peptides in cancers (201, 202).

The definitive molecular explanation(s) of the different extraintestinal expression patterns for CCK, gastrin, and other gut hormones remains to be found. Part of the explanation will have to be sought in the transcriptional regulation. Accordingly, the promoters of the otherwise homologous gastrin and CCK genes display marked differences in number and types of regulatory sites. The CCK gene promoter contains unusually many regulatory sites, which moreover have been exceedingly well preserved during evolution (174, 206, 256). Hence, the restrictive expression of the CCK gene appears to be a carefully and highly differentiated process conserved over several hundred million years. In contrast, the gastrin gene promoter has less well-preserved regulatory elements (27, 38, 119, 206, 256), which may contribute to explain the more promiscuous expression.

#### D. Cell-Specific Prohormone Processing

Cell-specific gene transcription is the very basis for the function of multicellular organisms. However, for gastrointestinal hormones and other regulatory peptides, gene and prohormone structures are often so complex and the posttranscriptional and posttranslational processing phases so elaborated (Figs. 4 and 6) that the phe-

notypic result of hormone gene transcription is unpredictable. It depends on the particular cell in which the gene is transcribed. One of the variable phases in the expression cascade is that of prohormone maturation, the posttranslational phase. Hence, the cellular equipment with processing enzymes and the necessary cofactors determines the structure of the particular prohormone product. This cell-specific processing of prohormones applies to all gastrointestinal hormones.

As in many physiological and clinical contexts, gastrin is the most extensively studied gastrointestinal hormone also with regard to molecular and cellular biology, including cell-specific prohormone processing. Almost every tissue in which progastrin is expressed has its own characteristic processing pattern. Four different patterns are shown in Fig. 7. For members of the gastrin family, the processing varies with respect to endoproteolytic processing and with respect to amino acid derivatizations such as tyrosyl sulfations and phenylalanyl amidations (Figs. 5–7). In this context, it is worth realizing that the different types of processing may influence each other, presumably by changing the affinity for the various processing intermediates as substrate for the processing enzymes. Thus tyrosyl sulfation, the earliest posttranslational modification for the gastrin family of prohormones (Fig. 6), increases the efficiency of the endoproteolytic cleavage (39, 40, 123). The more efficient the endoproteolytic cleavage is (i.e., the shorter the peptide substrates), the more efficient is the COOH-terminal amidation process (J. R. Bundgaard and J. F. Rehfeld, unpublished data), which is the final modification, decisive for receptor binding and bioactivity.

Recently, much attention has been devoted to cells that express and process progastrin, but only to glycine-extended gastrins. Glycine-extended gastrins are the immediate precursors of the carboxyamidated gastrins in the normal processing pathway (Fig. 6). Until recently, amidated gastrins have been considered to be the only bioactive forms of gastrin. A few years ago it was, however, shown that epithelial cells in colorectal carcinomas synthesize glycine-extended gastrins but no amidated gastrin (48, 173, 221), apparently because the cells do not express the amidation enzyme complex. This observation raised the question of whether glycine-extended gastrins in spite of their lack of effect on gastric acid secretion (105) might act as local, autocrine growth factors. Some evidence has been reported in favor of this idea. Thus there are reports of trophic effects of glycine-extended gastrins on AR4-2J and Swiss 3T3 cells (125, 172, 215, 242). Until the growth-mediating receptor specific for glycine-extended peptides has been structurally identified however, it is probably too early to take glycine-extended peptides for growth factors. If, however, the receptor eventually is identified, it would add a new dimension to the phenomenon of cell-specific processing for the many amidated gut hormones and regulatory peptides.

As already discussed, cell-specific processing reflects one of the possibilities for utilization of one gene for synthesis of several bioactive peptides. In addition to the cell-specific ways in which prohormones belonging to the gastrin family are processed (Fig. 7), prohormones such as proglucagon that contain different hormone sequences may be processed by a different principle. Similarly, most prohormones identified in gut endocrinology undergo some kind of cell-specific processing.

### E. Cell-Specific Peptide Release

To understand the specific effects of the different gastrointestinal peptides, it is necessary to realize that the different types of cells in which the respective genes are expressed also release the peptides in different ways (Fig. 8). As mentioned in section I, secretion of gastrointestinal hormones was until approximately 20 years ago supposed to be endocrine only, i.e., secretory granules from the endocrine cells in the gastrointestinal mucosa emptied their peptides in surrounding capillaries after appropriate stimulations.

In contrast to the systemic release of gastrointestinal hormones from classical endocrine cells, three alternative routes of secretion to neighboring cells and one to the secretory cell itself have been discovered during the last 20 years (Fig. 8). First, the peptides synthesized in neurons are released from synaptosomal vesicles in the nerve terminals to the receptors of adjacent target cells, i.e., neurotransmitter or neurocrine release. It is possible that

a spillover of gut hormonal peptides released from peripheral neurons may be transported via blood, analogous to other extraintestinal neuropeptides. It is also possible that some peptidergic neurons expressing gut hormonal peptides like some hypothalamopituitary neurons release the peptides directly to blood vessels. Second, as suggested by Feyrter (74) already in the 1930s and in the wake of the discovery of the morphological substrate for paracrine secretion (i.e., somatostatin cells that project short cytoplasmatic processes to neighboring cells, Ref. 138), it has been shown that there are paracrine cells for other gut hormones in the small intestinal mucosa (136). These cells carry peptidergic granules through cytoplasmatic extensions to specific target cells in the neighborhood. Paracrine cells can be conceived as hybrids of classical endocrine cells and neurons. It is consequently possible that also a local spillover of peptides from paracrine cells may reach the circulation.

Self-stimulation or autocrine secretion was described first by Sporn and Todaro (226) and subsequently substantiated by others (53, 111, 225). By autocrine secretion cells stimulate their own growth. Trophic peptides bind to specific receptors in the membranes of cells in which they are also synthesized (Fig. 8). Autocrine secretion is supposed to play a decisive role in tumor and cancer development (225). The first example was small-cell bronchogenic cancers, which were stimulated by autocrine secretion of GRP (53, 145). Recent evidence suggests that the growth of certain cultured bronchial carcinoma cells (213), colon carcinoma cells (111, 112), gastric carcinoma cells (254), and pancreatic tumor cells (19, 214, 253) is stimulated by autocrine secretion of gastrin and that growth of certain human pancreatic cancers and cell lines is stimulated by CCK peptides (100, 219, 220).

Cellular release of gastrointestinal peptides also occurs in a fifth way that cannot be ascribed to any of the four above-mentioned ways (Fig. 8). As already mentioned, spermatogenic cells in mammals other than humans express the CCK gene (180), whereas human spermatogenic cells express the gastrin gene (208). In spermatozoans, CCK or gastrin peptides are fully carboxyamidated and concentrated in the acrosome. In accordance with the acrosomal reaction, the peptides are released from the spermatozoan by contact with the jelly coat of the egg, and subsequently bound to receptors in the egg membrane. So far, CCK or gastrin receptors have not yet been demonstrated in mammalian egg cell membranes, but availability of probes for the CCK and gastrin receptors (132, 252) makes a search for them feasible in a foreseeable future. Acrosomal release may prove to be one of the most important mechanisms of secretion for gut peptides if fertilization of the egg (i.e., the beginning of life in an ontogenetic sense) turns out to require such peptides. We suggest that the release

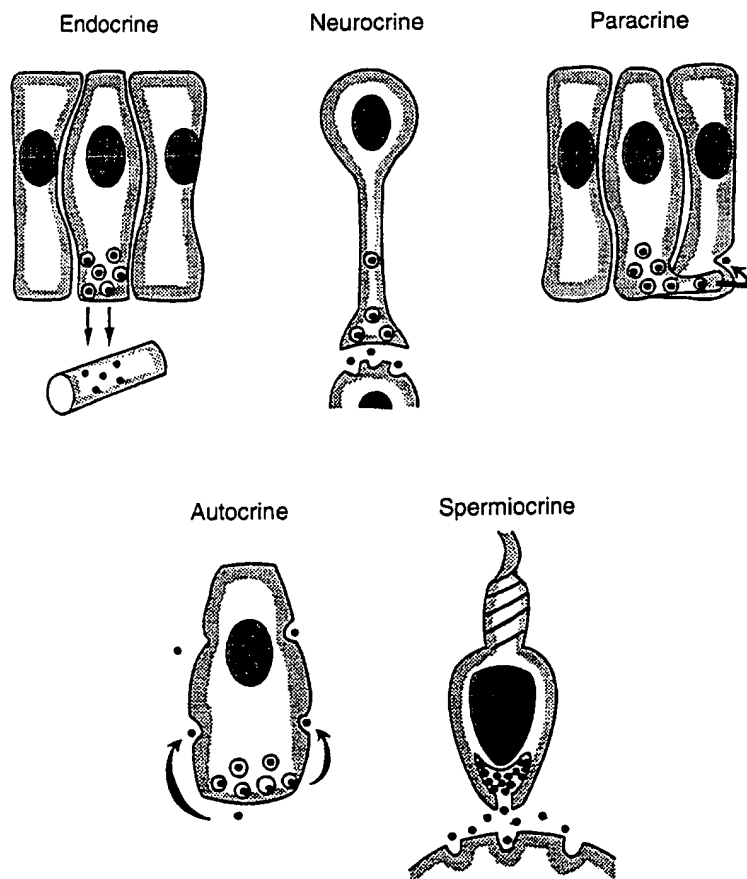


FIG. 8. Five types of cell-specific release of regulatory gut peptides: 1) endocrine release to capillaries from classic endocrine cells in gastrointestinal mucosa; 2) neurocrine release from central or peripheral neurons to synaptic cleft; 3) paracrine release to neighboring cells through short cellular processes; 4) paracrine cells are often of closed type, which do not reach intestinal lumen, and auto-crine release to receptors on membrane of same cell that synthesizes peptides; and 5) spermiocrine release from acrosomal granule of spermatozoans to receptors on egg cell membrane.

of bioactive peptides from acrosomal granules is named spermiocrine release (Fig. 8).

#### IV. PERSPECTIVE

Gastrointestinal endocrinology has over the last quarter of this century developed from a small appendix to endocrinology consisting of three distinct peptides with local secretagogue functions, to a biological discipline of its own comprising a multitude of more than 100 bioactive peptides, which are expressed in a controlled cell-specific manner all over the body. The peptides participate in intercellular regulation from local control of growth and differentiation to acute systemic effects. Thus travelers on the journey beginning in the early 1970s have witnessed a revolution that has changed fundamental concepts and opened wide perspectives in physiology and pathophysiology.

Today, gastrointestinal hormones must be viewed as highly conserved general intercellular messengers. There are no obvious boundaries between their role in food intake and digestion and their function in other bodily regu-

lations. It is possible and even likely that most regulatory peptides (hormones, neurotransmitters, growth factors, cytokines) at least at some stage in the phylo- or ontogenetic development are expressed in the gut. Hence, the development of gastrointestinal endocrinology as depicted in Figure 1 may on the one hand continue its exponential growth with a broad definition of regulatory peptides. On the other hand, such extension almost deprives the concept of gastrointestinal endocrinology of its meaning, and that is exactly what it is all about. Gastrointestinal hormones should be viewed not only as local hormones of interest for digestive physiologists and clinical gastroenterologists. Instead, gastrointestinal hormones are integrated participants in the coordination and regulation of many or most functions in vertebrates, and perhaps also in invertebrates. Thus it is in retrospect not surprising that classic gut hormones today are studied not only in physiology, pharmacology, biochemistry, and cell biology, but also by psychologists, psychiatrists, zoologists, molecular oncologists, diabetologists, and others.

The most important developments to come for gut hormone research are likely to occur in basic cell biology with applications to clinical oncology and in neurobiology

with application to psychiatry. The technology in cell biology and neurobiology is at present so powerful that pioneers from the 1960s and early 1970s can hope to witness measurement of some element in the gastrointestinal hormone systems (genes, precursors, or receptors) in diagnosis, classification, and monitoring of treatment of major cancers and common psychiatric diseases.

The skillful and patient secretarial assistance of Gitte Runge Hansen is gratefully acknowledged.

This review is based on experience from three decades filled with excitement in gut hormone laboratories in Denmark (Copenhagen and Århus) and during visits to Sweden (Lund and Stockholm), Germany (Göttingen), England (Cambridge and Liverpool), and the United States (Ann Arbor, Boston, Gainesville, Los Angeles, and New York).

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